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Biodiesel Production using Lipase from Oil Palm Fruit as A Catalyst

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Abstract

This study aimed to extract and characterize lipase from oil palm after 0-240 h of harvesting. In addition, the application of lipase as catalyst for biodiesel production was also evaluated. Lipase was extracted and purified by Tris-base buffer (pH 8.0) and the aqueous two phase system (ATPS), respectively. The highest protein at 1.96 mg/gat was obtained from oil palm fruit at 0 h of harvesting. However, the highest lipase activity at 0.98 Units (1.38 Unit/mg protein) was achieved from palm oil after 120 h of harvesting. Afterwards, lipase was collected and purified by ATPS using PEG 1000 under the variation of salts. After purification, lipase activity was increased significantly to 4.76 Unit/mg protein using PEG and NaH_2PO_4. Therefore, purified lipase was utilized as catalyst for biodiesel production using the transesterification method and the partial properties of biodiesel from lipase were also determined. The biodiesel from lipase had an acid value and free fatty acid content at 0.45 mg/g KOH and 0.21%, respectively. The properties of biodiesel were also compared with commercial biodiesel. Interestingly, the acid value and free fatty acid content of biodiesel from lipase were not significantly different from commercial biodiesel and it also passed Thailand's fuel standards.

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Keywords: Aqueous two-phase systems, ATPS, Biodiesel, Lipase, Transesterification

1. Introduction

Lipase is the enzyme that was a catalyst in the process of transesterification. Normally, microbial lipases have been used for biodiesel because of their resistance to high temperature and cheap cost for extraction and purification [1]. However, high lipase activity from oil palm has also been reported. Thailand is one of the world leading producers of oil palm. Due to increasing fuel demand, the research

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and development for novel lipase has been a challenge. Therefore, palm oil is the most suitable source for lipase extraction [2-4].

Biodiesel, consisting of fatty acid monoesters derived from renewable feedstocks, is one of the most promising alternative fuels. It is biodegradable, non-toxic, almost sulfur-free, and can be used alone or blended with conventional petrodiesel in unmodified diesel engines [5]. Commercially, biodiesel is most often produced by transesterification of vegetable oils with short chain alcohols using alkaline catalysts. The alkali-catalyzed process has the advantages of short reaction time, high yield, and low cost for the catalysts. However, this process often requires high quality food-grade vegetable oils with low level of free fatty acids (FFA) to prevent saponification, which leads to difficult glycerol separation and low ester conversion rate [6]. Using lipases as catalysts can overcome many aforementioned drawbacks of the alkali-catalyzed process [7]. The enzymatic process can utilize low quality feedstocks with high levels of FFA because FFAs can be directly converted to biodiesel via lipase-catalyzed esterification. It also requires less energy input and the glycerol byproduct is easier to separate. Even with many desirable properties, the enzymatic process has very limited commercial success mainly due to the high cost of lipase. [8]. The objective of this study aimed to extract and characterize lipase from oil palm after 0-240 h after harvesting. In addition, the application of lipase as a catalyst for biodiesel production was also evaluated.

2. Research methodology

2.1 Extraction of lipase

Oil Palm fruit after 0-240 h of harvested were collected from Phatthalung (Thailand). Lipase was firstly extracted by 50 mM Tris-base buffer (pH 8.0). The extraction was done at the ratio of an oil palm fruit and buffer solution at 1: 2 (w/v). The supernatant was collected by centrifugation at 10,000 rpm for 30 minutes at 4^{o}C. The supernatant was kept at 4^{o}C until used.

2.2 *Determination of total protein*

The Determination of protein was followed by Lowry method [9].

2.3 Determination of lipase activity

The activity of lipase was determined by adding 200 µl samples in 0.1 mM Tris-HCl buffer (pH 8.0) 2.45 ml containing 0.15 M NaCl and 0.5% Triton X- 100. The determination was operated under 40ºC for 5 minutes, then add 50 mM *p*-nitrophenylpalmitate 200µl. The sample was determined for the absorbance at 410 nm compared with *p*-nitrophenol standard curve at concentrations ranging from 0 - 1.0 μg.

2.4 Purification of lipase from oil palm fruit using aqueous two-phase systems

For ATPS method, polyethylene glycol (PEG) 1000 was utilized with the variation of salts. Crude enzyme (1 g) was firstly mixed with 5 g of distilled water. The sample was loaded into ATPS system. Lipase was purified in a bottom layer (salt solution). Therefore, purified lipase was collected and characterized.

2.5 The Production of biodiesel using lipase as a catalyzt

The purified lipase was mixed with vegetable oil and methanol. The ratio of oil, methanol and lipase is 10: 2: 1. The transesterification was operated under room temperature for 3 h. Therefore, only methyl ester was collected and characterized.

2.6 The analytical method

Biodiesel from lipase was characterized for viscosity, acid value and free fatty acid content. The viscosity was analyzed by the gravity method. In addition, acid value and free fatty acid content were determined by titration method.

3. Results and Discussion

3.1 The extraction of lipase from oil palm fruit after harvested

The highest protein at 1.96 mg/gat was obtained from oil palm fruit at 0 h of harvesting. However, the highest lipase activity at 0.98 Units (1.38 Unit/mg protein) was achieved from palm oil after 120 h of harvesting (Table 1).

Table 1 The total amount of protein (mg/g), total amount of lipase (units) and specific lipase activity (unit/mg protein) of extracted lipase from palm oil after 0-240 h of harvested.

Time (h)	**Total protein)mg/g)**	**Total lipase (units)**	**Specific Activity (unit/mg protein)**
0	1.96±0.01	0.59	0.30
24	0.98±0.01	0.61	0.63
48	0.86±0.01	0.64	0.75
72	0.81±0.01	0.77	0.95
96	0.76±0.01	0.87	1.15
120	0.71±0.01	0.98	1.38
144	0.68±0.01	0.66	1.06
168	0.61±0.01	0.63	1.03
192	0.56±0.00	0.58	1.03
216	0.51±0.01	0.49	0.96
240	0.46±0.01	0.35	0.76

Afterwards, lipase was taken and purified by ATPS using PEG 1000 under the variation of salts. After purification, lipase activity was increased significantly to 4.76 Unit/mg protein using PEG and NaH_2PO_4 (Table 2). The partition of lipase strongly depended on the type and concentration of salts. The recovery of lipase increased with 15% NaH_2PO_4. The high V_R of ATPS would allow faster processing of the extraction process. Most of the system used in this study showed the high V_R value (0.88-1.09). In addition, K_P value of all ATPs system was in the ranges of 0.61-1.08 with the average around 0.89. The highest K_p (1.02) was found in the system of 20%PEG1000-15% NaH_2PO_4. The K_p value indicated the distribution of the protein in ATPs. The high K_p value showed that most of the protein in sample partitioned in only the top phase. Furthermore, the K_E obtained from optimum systems was also higher than 5, indicating that only the target enzymes were partitioning to the top phase.

Table 2 Specific activity of extracted lipase after purified by aqueous two-phase systems using 20%PEG1000 as polymer phase.

Phase transition	V_R[a]	K_P[b]	K_E[c]	Specific activity (units/mg protein)	
				Top	Bottom
15% NaH_2PO_4	1.90	0.84	6.00	4.76	0.37
15% $(NH_4)_2SO_4$	1.17	1.08	5.82	3.62	0.53
15% $MgSO_4$	2.43	0.61	0.34	3.57	2.48
15% K_2HPO_4	0.92	1.00	1.89	4.05	2.31
15% Na_3C_6H5O	1.09	0.76	3.28	3.70	0.81
15% Na_2SO_4	0.88	1.02	1.08	4.03	3.29

[a]V_R: A phase volume ratio; [b]K_P: Protein partitioning coefficient; [c]K_E: Enzyme partitioning coefficient

Ooi et al. [10] reported that lipase production from *B. pseudomallei* was obtained in the top phase of PEG 8000/Dextran ATPS. The *B. pseudomallei* cell and other protein contaminants were exclusively partitioned to the bottom phase. The best conditions for the extractive microbial fermentation was performed in an ATPS comprising 9.6% (w/w) of PEG 8000 and 1.0% (w/w) of Dextran T500. High yield of 92.1% was achieved in the single batch of ATPS extractive fermentation. However, the extraction and purification of lipase from oil palm fruit have not been widely demonstrated.

3.2 The production of biodiesel

3.2.1 Analysis viscosity

Therefore, purified lipase was utilized as a catalyst for biodiesel production using the transesterification method and the partial properties of biodiesel from lipase were also determined. Biodiesel from lipase was chacterized for viscosity, acid value and free fatty acid content. The viscosity was analyzed by the gravity method (Table 3).

Table 3 The viscosity of biodiesel from lipase compared with commercial biodiesel and oil.

substance	Time (sec)	Relatively viscosities Compared with distilled water
Biodiesel catalyzed by bases	8	1.6
Biodiesel catalyzed by enzymes	14	2.8
Oil (blank)	70	14
Thailand fuel standard		3.5-5.0[a]

[a]Biodiesel (methyl ester) standards and specification premium by MOE specification and customer special requirement (Thailand)

In addition, the acid value and free fatty acid content were determined by titration method (Table 4). Interestingly, the acid value and free fatty acid content of biodiesel from lipase were not significantly different from commercial biodiesel and it also passed Thailand's fuel standards.

Table 4 The acids value and free fatty acids content of biodiesel from lipase compared with Thailand's fuel standards.

Fuel property	Limit[a]	Product biodiesel
Acid value (mg/g)	< 0.5	0.45
Free fatty acid (%)	< 3%	0.21

[a]Biodiesel (methyl ester) standards and specification premium by MOE specification and customer special requirement (Thailand)

4. Conclusion

Lipase was extracted and characterized from oil palm after 0-240 h of harvesting. The highest protein (1.96 mg/gat) and lipase (1.38 units/mg protein) were obtained from oil palm fruit after 0 and 120 h of harvesting, respectively. After purification by ATPS, lipase activity was increased significantly to 4.76 Unit/mg proteins. Purified lipase was utilized as a catalyst for biodiesel production and the partial properties of biodiesel from lipase were also determined. The biodiesel from lipase had an acid value and free fatty acid content at 0.45 mg/g KOH and 0.21%, respectively.

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